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# THE EFFECT OF ENVIRONMENTAL TEMPERATURE ON LETHALITY OF ENDOTOXIN AND ITS EFFECT ON BODY TEMPERATURE IN MICE

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# THE EFFECT OF ENVIRONMENTAL TEMPERATURE ON LETHALITY OF ENDOTOXIN AND ITS EFFECT ON BODY TEMPERATURE IN MICE

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## FOREWORD

This is a final report prepared under contract AF 41(609)-1764 (Project 8241, Task 824101) with the Department of Biology, Bryn Mawr College, Bryn Mawr, Pennsylvania. This report covers research carried on from 1 March 1965 to 31 May 1965. Air Force program monitor is Mr. Robert Becker, ALRA, Arctic Aeromedical Laboratory.

This technical report has been reviewed and is approved.

HORACE F. DRURY
Director of Research

## ABSTRACT

Acute exposure of mice to an environmental temperature of either 5° C or 37° C reduced the $LD_{50}$ of a crude Serratia marcescens endotoxin from a high of 2300 µg in mice housed at 30° C to an amount less than 40 µg. At 15° C or 32° C, the $LD_{50}$ was, respectively, 880 µg and 550 µg, while at 25° C it was 1200 µg. Control animals placed at each of these temperatures were able to maintain normothermia except for those at the high and low extremes where they became slightly hyperthermic and hypothermic. Following an injection of either twice the $LD_{50}$ or a dose of 1000 µg, the thermoregulatory ability was upset at all temperatures except 30° C. Mice at temperatures below 30° C became progressively more hypothermic as the environment was increasingly cold and vice versa at higher temperatures. It is believed that endotoxin sensitizes mice to heat and cold rather than these temperatures sensitizing to endotoxin. After one week of acclimatization at 5° C or 37° C, the $LD_{50}$ of endotoxin increased, respectively, to 790 µg and 260 µg. Inducibility of the liver enzyme tryptophan pyrrolase, believed to play a role in an animal's response to endotoxin, was evaluated at each environmental temperature. Only at the extremes was it suppressed.

## NOTICES

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This research was conducted in accordance with the "Principles of Laboratory Animal Care" of the National Society for Medical Research.

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# I

# INTRODUCTION

Previous reports from this laboratory (1, 2, 3) have shown that exposure of mice to an environmental temperature of 5° C sensitizes to the lethal effects of bacterial endotoxin. Similarly, exposure to a temperature of 37° C sensitizes to the same degree to endotoxin (1, 4). The basis of this effect remains uncertain. It is clear, however, that animals at these extremes of temperature die more rapidly than mice at more conventional environmental temperatures, and appear to die "atypically" as far as endotoxin poisoning is concerned. The present report is designed to bring into clearer perspective results previously described.

Animals were exposed to several temperatures intermediate between 5° C and 37° C. The change in their body temperature was followed with and without an injection of endotoxin proportional to the $LD_{50}$ and also to a fixed amount of endotoxin given to animals housed at all temperatures. It becomes apparent from these observations that one of the earliest and most acute effects of endotoxin is to upset the thermoregulatory mechanism of the mouse. This effect tends to disappear with a week of acclimatization at each of the extremes of temperature.

# II

# METHODS

## Endotoxins

A washed lyophilized whole cell preparation of Serratia marcescens (generously supplied by Merck and Co., Rahway, N. J.) was used in most of the experiments. Results obtained with this crude material were repeated in part with a bovine extract of Escherichia coli (generously supplied by Dr. C. W. Dewitt, Upjohn Co., Kalamazoo, Mich.). Each preparation was suspended in nonpyrogenic isotonic sodium chloride solution (Baxter Laboratories, Morton Grove, Ill.) and injected intraperitoneally at the desired dose level contained in a volume of 0.5 ml.

## Body Temperature Measurements

Body temperatures were measured with a telethermometer (Yellow Springs Instrument Co., Model 47 TA) using the small animal rectal probe (YSI No. 402). The probe was inserted for a distance of 1 cm and the

temperature was read 30 seconds later. Each value presented is the average of 10 separate measurements on individual animals. Since the major interest was in the effect of acute exposure to environmental temperature, body temperatures were recorded only for the first three hours. Injections of endotoxin were given at time zero. Body temperatures were recorded at 30 minute intervals for the first two hours and again at three hours.

## Cortisone

Cortisone prepared in stabilized aqueous suspension as cortisone acetate was administered subcutaneously in 5 mg amounts. All injections were given at approximately the same time as the endotoxin or else were given alone.

## Temperature Control

A Labline Modulab was employed for all exposures to temperature. This environmental room can be set at any desired temperature from 5° C to 45° C and holds to within ± 0.5° C throughout the entire temperature range. The relative humidity was not controlled. Animals were singly housed and exposed without bedding as described by Previte and Berry (1).

## Tryptophan Pyrrolase Assays

The method of Knox and Auerbach (5) as modified for mice at Bryn Mawr College (6) was used in determining the tryptophan pyrrolase activity in livers of experimental and control mice.

## Mice

A Swiss-Webster strain of albino mice was purchased from Dierolf Farms, Boyertown, Pa. Animals were delivered weekly at a weight of approximately 18 gm. For the first three days, they were put on a solution of antibiotic (Polyotic, American Cyanamid Co.) in an effort to control and reduce variability due to latent and chronic intestinal infections. Following this treatment, they were placed on tap water for the subsequent four days or longer before being used experimentally. Female mice were employed exclusively and they were used when their body weight reached 22 gm ± 1 gm. Prior to experimental manipulations they were fed a pathogen-free laboratory diet (Wilhoite-Price, Frederick, Md.). Pathogen-free pellets and water were available ad libitum.

## III

## RESULTS

### Effect of Environmental Temperature on the Lethal Effect of Bacterial Endotoxin

As indicated by the solid line in Figure 1, the $LD_{50}$ of lyophilized whole cells of Serratia marcescens varied significantly with environmental temperature. The minimum dose occurred at 5° C (34 μg as determined with 110 mice) and at 37° C (37.6 μg as calculated with 70 mice). These values, and all others, were calculated by the method of Reed and Muench (7). Raising the environmental temperature from 5° C to 15° C increased the $LD_{50}$ approximately 25-fold (880 μg, as calculated with 80 mice). A further increase to 25° C raised the $LD_{50}$ to 1200 μg (calculated with 40 mice) and, quite dramatically, in going from 25° C to 30° C the $LD_{50}$ approximately doubled (2300 μg, determined with 100 mice). An environmental temperature of 32° C reduced the $LD_{50}$ to approximately one-fourth the value at 30° C (560 μg, as determined with 50 animals). It is quite apparent that the critical change in temperature occurs between 25° C and 32° C. The temperature at which the animals are most refractory is 30° C and one might suspect that this is the temperature at which endotoxin exerts its true primary toxicity rather than having secondary effects intervene to alter the survival of the animals. Similar results were obtained when purified E. coli lipopolysaccharide was used. The results are plotted as the dashed line in Figure 1.

### Effect of Environmental Temperature on the Body Temperature of Normal Mice

In an earlier publication by Previte and Berry (2), it was reported that animals placed at 5° C show during the first 5 to 6 hours a drop in body temperature of approximately 2° C and, by 12 hours, a temperature that has returned to the normal range. Apparently, therefore, the thermoregulatory capacity of mice is capable of coping with this temperature stress. Since animals at low temperature, when given the approximate $LD_{50}$ of endotoxin, begin to die within a matter of four hours, it becomes important to examine the early change in body temperature that is associated with exposure to the different environmental temperatures. These results are presented graphically in Figure 2. Several facts merit comment. The initial body temperature of animals at time zero shows a range of approximately 1° C. This is the result of the well-known variation in body temperature that can be made to behave cyclically if animals are subjected to proper control conditions (8). In an effort to keep the contribution of circadian rhythms to the variability of results to a minimum, all "zero time" body temperature measurements were made at 11:00 A.M. ± 30 minutes. In mice at 5° C, an abrupt drop in body

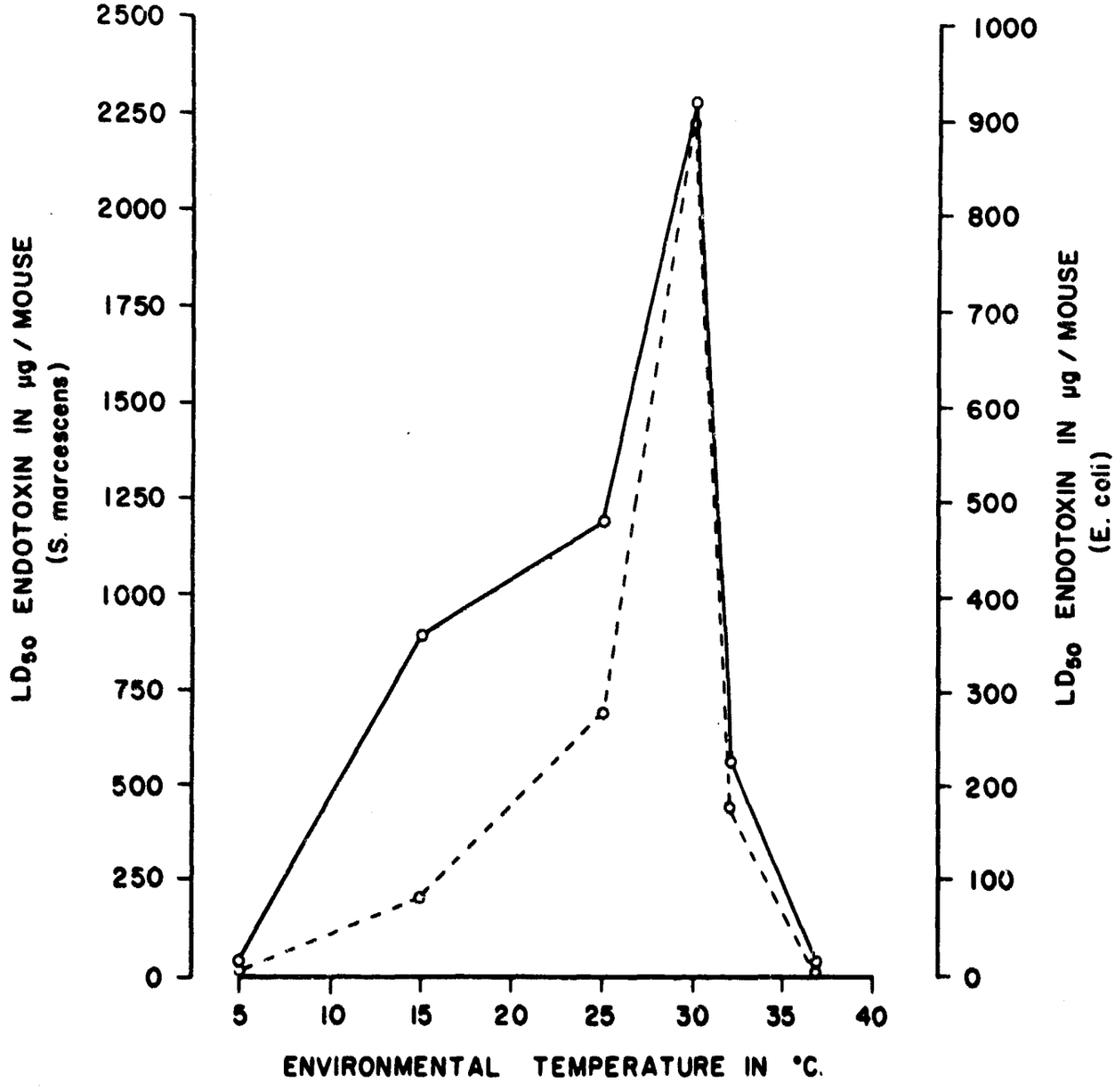


FIGURE 1

The $LD_{50}$ (in micrograms per mouse) of crude Serratia marcescens endotoxin in mice exposed individually to the designated environmental temperatures (solid line). The dashed line presents similar date for purified Escherichia coli lipopolysaccharide.

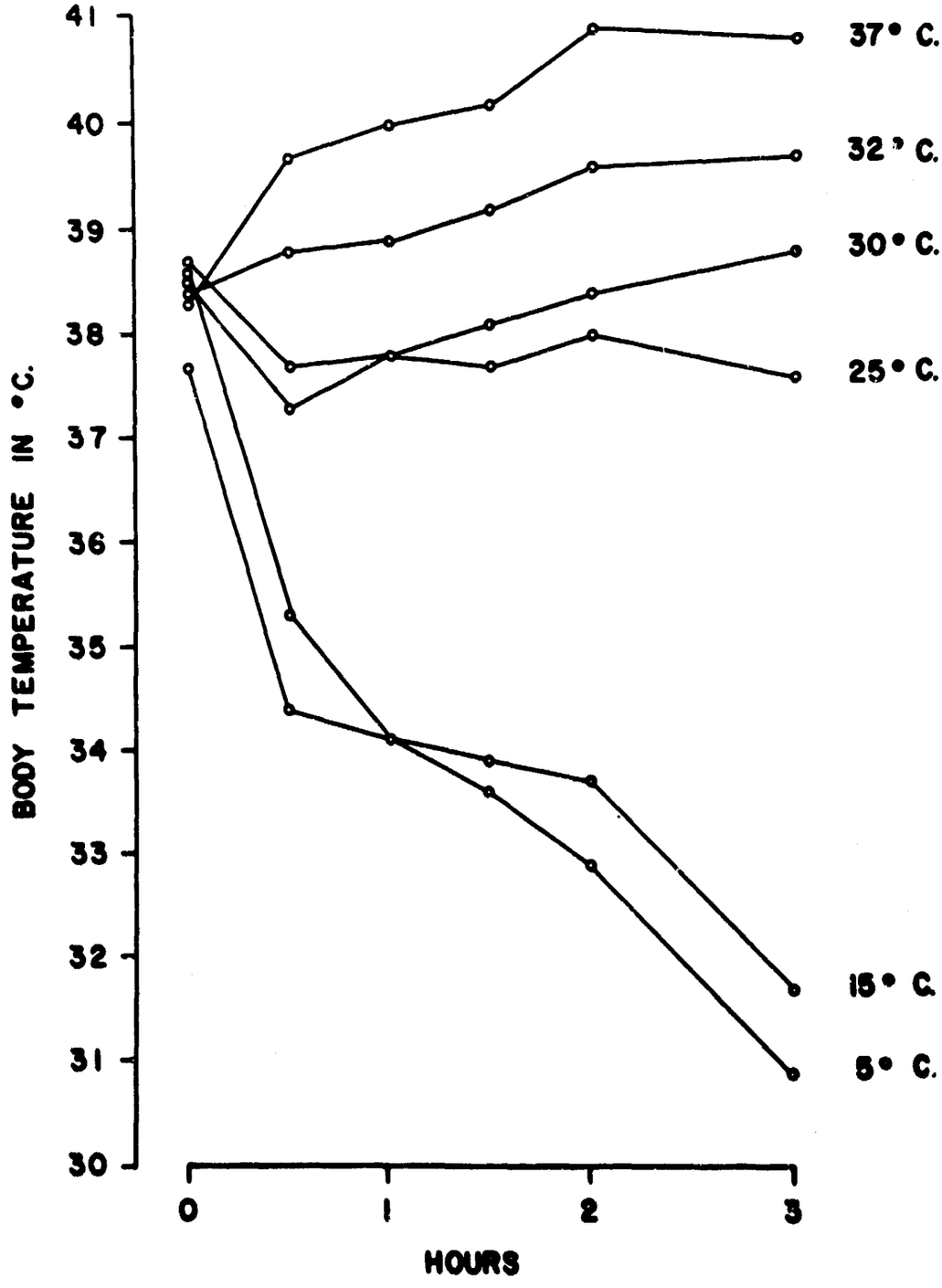


FIGURE 2

Body temperature of mice during the first three hours of individual exposure to the designated environmental temperatures. Each animal received at time O an intraperitoneal injection of 0.5 ml of nonpyrogenic saline. Each value is the average for 10 animals.

temperature occurs during the first 30 minutes. This is followed by a more or less steady decline of approximately 1° C in the succeeding 2-1/2 hour period. A similar but less severe drop in body temperature occurs in mice placed at 15° C. Animals at 25° C, 30° C, and 32° C show only a small variation in body temperature but, interestingly enough, the temperatures of the animals are distributed in a manner equivalent to the environment; that is the lowest temperatures are at 25° C, the highest at 32° C. In addition, the animals at 32° C show what might be called a slight elevation in temperature, at least compared with those at 30° C and at 25° C. This difference appears even though the specific points are not statistically significant. At 37° C, however, there is a clear development of hyperthermia. This occurs during the first one-hour period and is then more or less regulated for the succeeding two hours.

Effect of Endotoxin on Body Temperature of Mice at Different Environmental Temperature

Two types of experiments were done. In the first, the body temperature of mice was followed during the initial three-hour period at each environmental temperature following an intraperitoneal injection of twice the $LD_{50}$ characteristic of that temperature. These results are presented graphically in Figure 3. At both 5° C and 15° C, there is a very pronounced drop in body temperature during the first 30-minute period. This is followed by a steady decline during the next 2-1/2 hours. Note that the body temperature at 15° C is lower than that at 5° C but the quantity of endotoxin is approximately 30 times greater and hence may be related to the effect. Some animals at each of these temperatures are near death at the end of the three-hour period. These results are in marked contrast to those observed at 25° C and at 30° C. At 25° C, the temperature nearest to that normally associated with "room temperature", one notes a drop in body temperature that occurs promptly and continues for the next 1-1/2 hours with a slight rise during the final hour. This hypothermia associated with endotoxin is a well-known response for the mouse. The effect at 25° C is in contrast to that at 30° C. Here, the range in body temperature is within normal limits and one may assume that endotoxin exerts little, if any, effect on body temperature of mice housed at 30° C. At 32° C, however, there is a clear and marked hyperthermia developing in these animals but one that is not as severe as that appearing in mice housed at 37° C. Animals exposed to 37° C have a body temperature that is probably incompatible with continued life because these animals begin to die at the end of three to four hours.

In order to make a different kind of comparison, the effect of endotoxin was determined when given in constant amount to mice then housed at different environmental temperatures. The results are presented graphically in Figure 4. Following an injection of 1000 μg of crude S. marcescens endotoxin, body temperature dropped more severely at 5° C than at 15° C, even though the difference is minor. The animals at 5° C and at 15° C began

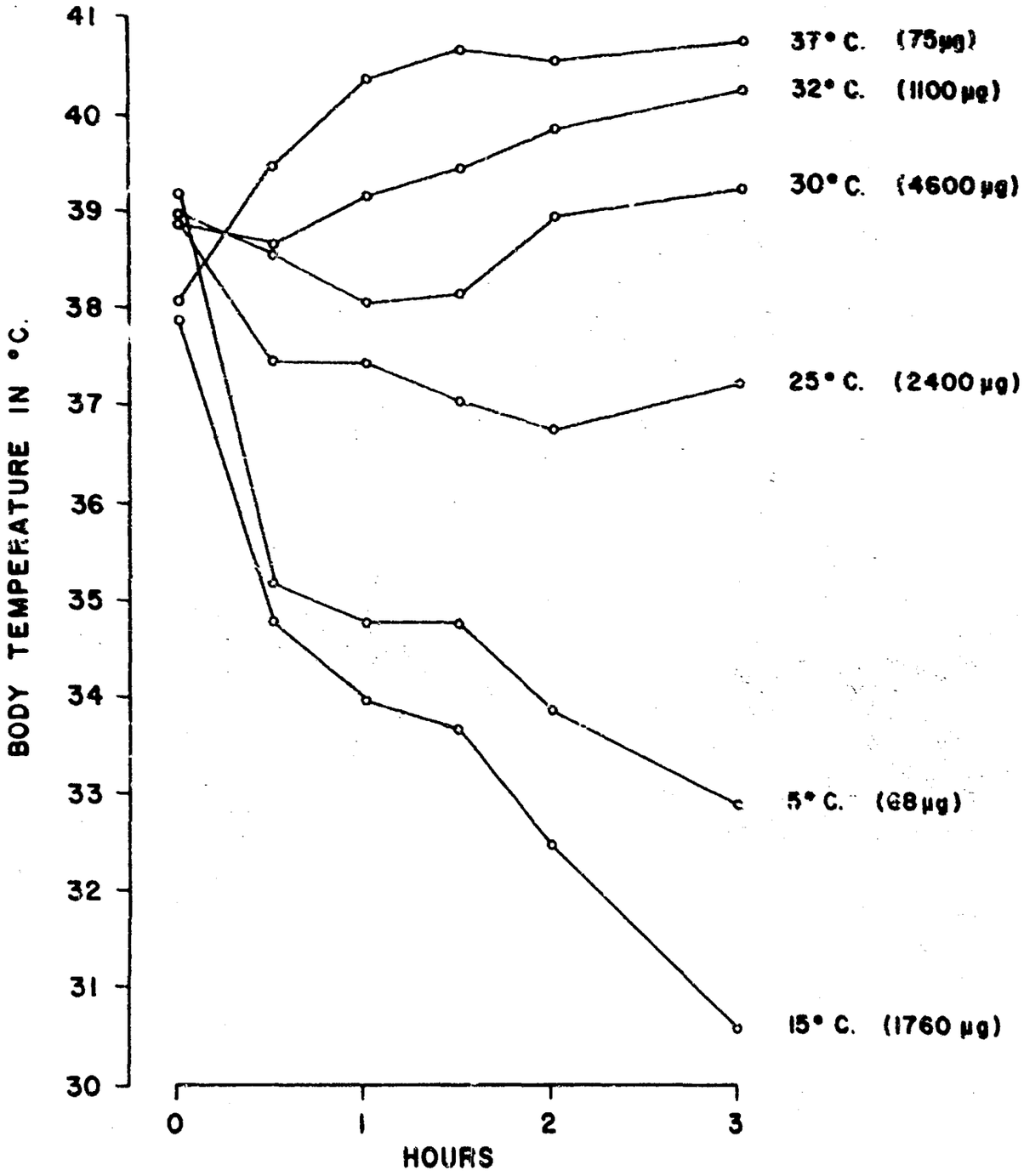


FIGURE 3

Body temperature of mice during the first three hours of individual exposure to the designated environmental temperatures. Each animal received at time O an intraperitoneal injection of 2 X $LD_{50}$ of Seratia marcescens endotoxin contained in 0.5 ml of nonpyrogenic saline. Each value is the average for 10 animals.

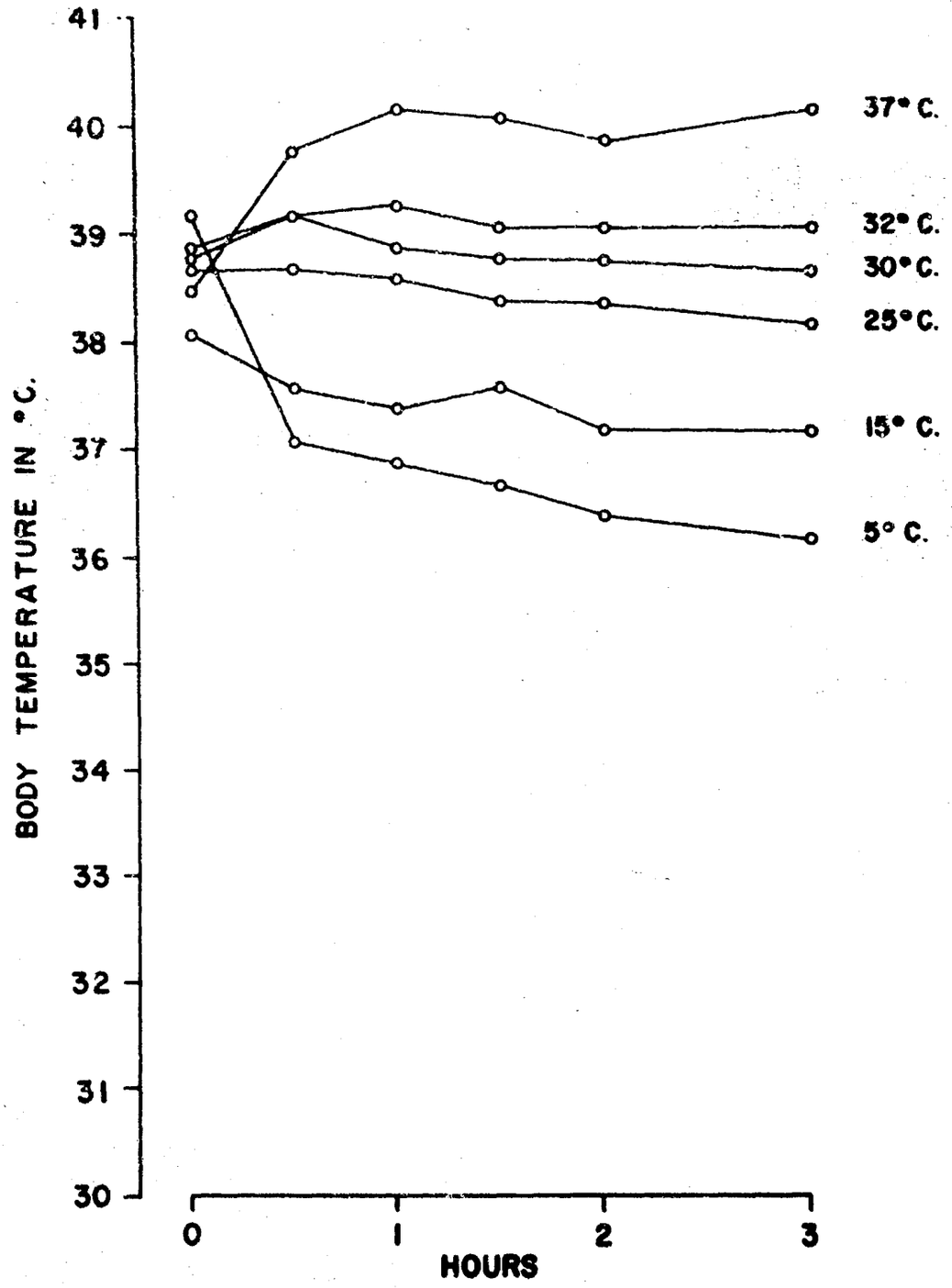


FIGURE 4

Body temperature of mice during the first three hours of individual exposure to the designated environmental temperatures. Each animal received at time O an intraperitoneal injection of 1000 μg of Serratia marcescens endotoxin contained in 0.5 ml of nonpyrogenic saline. Each value is the average for 10 animals.

to die between three and four hours postinjection, possibly of hypothermia. At 25° C and 30° C, where the dose of endotoxin is less than the $LD_{50}$, little temperature change was observed. At 32° C, the dose of endotoxin is approximately twice the $LD_{50}$ and the result was similar to that shown in Figure 3. At 37° C, the dose is 30 times the $LD_{50}$ and here there was a prompt rise in body temperature to a value that may approximate the maximum obtainable in mice. The animals began to die within the three-hour period, possibly of hyperthermia.

The data of Figures 3 and 4 raise a question of whether endotoxin in mice housed at 25° C causes a hypothermia about as severe as that seen at 5° C and 15° C but at a slower rate. This did not occur 24 hours after the injection of the $LD_{50}$ of endotoxin (1200 µg S. marcescens), a time when some of the mice had died. The average body temperature of 8 poisoned mice was 38.1° C and that of 10 controls was 37.9° C. Apparently, therefore, endotoxin can cause death of mice without the changes in body temperature seen in mice at other environmental temperatures.

## Effect of Environmental Temperature on Induction of Tryptophan Pyrrolase Following Cortisone Administration

Since there is accumulating evidence for a relationship between endotoxin poisoning and the activity of the liver enzyme tryptophan pyrrolase, the results summarized in Table I are presented. These data show the effect of environmental temperature on the level of enzyme activity and its inducibility following an injection of cortisone acetate. This enzyme is known to require hematin as a cofactor (9). Under normal conditions, only about half of the total enzyme present is in active form, i.e. is present as holoenzyme (3), while the remainder exists as apoenzyme. If one assumes that the assay without addition of cofactor (hematin) reveals most faithfully the level of activity that exists in vivo, then interesting results are made obvious in the data of Table I. As the headings of the Table suggest, two sets of values are presented for control mice and for mice four hours following an injection of cortisone. The activity of enzyme with heme and without heme is given under both conditions. The most evident relationship here is the effect of temperature in control animals on enzyme activity without the addition of heme. The highest activity is that seen at 5° C. This activity declines progressively to the minimum value at 30° C and then rises towards 37° C. The value shown at 30° C, the temperature at which mice are apparently minimally stressed, is very nearly the same as that reported in an earlier publication for enzyme activity in adrenalectomized mice (6). The approximate doubling of activity between 30° C and 5° C suggests that adrenal activity is stimulated by low temperature stress. By this token there would be either less stress at 37° C or, for some reason, less ability to synthesize enzyme. The other point suggested by these data is the nearly constant value of enxyme activity in mice with heme at all temperatures except that at 5° C, which again suggests

TABLE I

Cortisone Induction of Liver Tryptophan Pyrrolase Activity in Mice Four Hours after Acute Exposure to Different Environmental Temperatures Each assay was carried out with and without the addition of the cofactor, heme. Each value is the mean + the standard error of the mean for the number of separate determinations shown in parentheses.

| Environmental Temperature | Tryptophan Pyrrolase Activity (μM Kynurenine/gm dry wt liver/hr) in | | | |
|---|---|---|---|---|
| | Control Mice Assayed | | Cortisone Injected Mice Assayed | |
| | Without heme | With heme | Without heme | With heme |
| 5°C | 14.5 ± 1.9 (7) | 27.3 ± 2.1 (7) | 15.9 ± 1.0 (7) | 35.3 ± 2.3 (7) |
| 15°C | 12.4 ± 1.1 (10) | 22.7 ± 2.4 (10) | 15.6 ± 0.8 (10) | 31.1 ± 2.5 (10) |
| 25°C | 10.0 ± 0.8 (12) | 22.3 ± 1.9 (12) | 21.6 ± 1.2 (12) | 40.8 ± 4.0 (11) |
| 30°C | 7.8 ± 0.5 (10) | 18.1 ± 1.6 (10) | 19.6 ± 0.9 (10) | 32.9 ± 1.4 (10) |
| 32°C | 8.9 ± 0.5 (10) | 23.3 ± 2.0 (10) | 24.0 ± 1.5 (10) | 43.3 ± 3.2 (10) |
| 37°C | 10.1 ± 0.8 (10) | 16.7 ± 1.6 (10) | 22.6 ± 1.7 (10) | 37.8 ± 3.9 (10) |

an augmentation in total synthesis of enzyme, possibly arising from endogenous enzyme activity, and an apparent suppression of total enzyme activity in control mice with heme at 37° C.

Values obtained four hours after cortisone injection also indicate some interesting relationships. At both 5° C and 15° C, there is no increase in enzyme activity in values measured without heme. Apparently, therefore, one can expect no increased activity in enzyme within the in vivo situation if

this value reflects the true condition. At all other temperatures enzyme activity without heme at least doubles four hours after cortisone when compared to the control value. Similarly, there is a marked induction following cortisone at all temperatures, as judged by the addition of heme. Whether the addition of heme is the significant value to look at remains uncertain. One would say, however, that there is little protection afforded animals at 5° C and 15° C following an injection of cortisone, as judged by inducibility of enzyme.

## Protective Effect of Cortisone at Different Environmental Temperatures

It has been well-established that cortisone protects mice against the lethal effects of bacterial endotoxins at the range of temperatures normally experienced in laboratories. It has also been found that cortisone administered to mice acutely exposed to 5° C has only varied and doubtful ability to protect (2, 3). This may be related to its lack of ability to induce tryptophan pyrrolase. At 37° C, there is some protective effect of cortisone. as shown by the data in Table II. Again, this protection appears to be dose dependent, as it was at 5° C. The reasons for this are not at all clear.

TABLE II

Protective Effect of Cortisone against Endotoxin Lethality in Mice Housed at a Temperature of 37° C.

| Dose of Endotoxin (S. marcescens) | Number Dead / Number Injected | | P Value |
|---|---|---|---|
| | Without Cortisone | With Cortisone | |
| 40 µg | 8/20 | 12/20 | N.S.* |
| 300 µg | 0/20 | 9/20 | P = .014 |

* N. S. = Not significant.

## Effect of Cortisone Singly and in Combination with Endotoxin on Body Temperature of Mice at Different Environmental Temperatures

As the family of curves presented in Figure 5 indicate, cortisone either alone or when administered with endotoxin has no effect on the change in body temperature accompanying exposure to 5° C and at this temperature there is

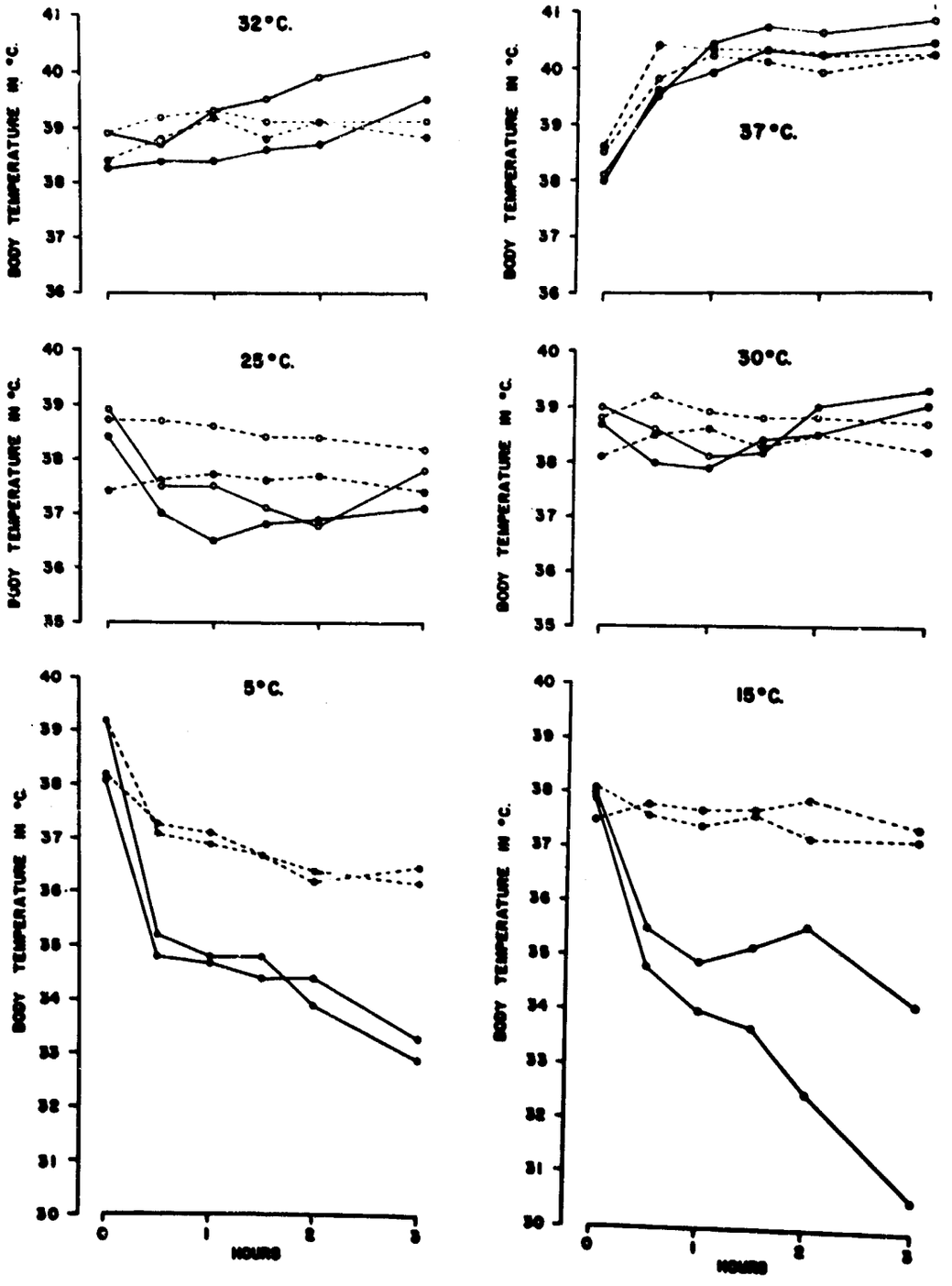


FIGURE 5

Body temperature of mice during the first three hours of individual exposure to the designated environmental temperatures. Each animal received at time O an injection of nonpyrogenic saline (open circles with dashed line), nonpyrogenic saline and cortisone (closed circles with dashed line), 2 X $LD_{50}$ of Serratia marcescens endotoxin (open circles with solid line), or 2 X $LD_{50}$ of endotoxin and cortisone (closed circles with solid line). The saline or endotoxin was given intraperitoneally and the cortisone subcutaneously. Each value is the average for 10 animals.

doubtful, if any, protective effect of cortisone. At 15° C, however, there is no effect of cortisone on control temperature but when cortisone is administered concurrently with twice the $LD_{50}$ of endotoxin, the body temperature does not fall to such a low temperature. This would apparently correlate with the ability of cortisone to protect these mice against the lethal effects of endotoxin. At all other temperatures cortisone has no effect.

The Effect of Acclimatization at Extreme Temperatures on the Lethal Effects of Bacterial Endotoxin

Mice that have been housed at 5° C for one week and then challenged with endotoxin show an $LD_{50}$ of 790 μg. This is more than 20 times the value in animals acutely exposed. This value of 790 μg is based on the Reed-Muench calculation (7), where 84 mice were used. Mice exposed to 37° C for one week undergo at least 50% mortality and here the change in $LD_{50}$ is not as pronounced but yet is highly significant. The $LD_{50}$ is 260 μg, as based on 20 mice. One of the characteristics of acclimatization would appear to be, therefore, an increased refractoriness of the thermoregulatory center of the animals to modification by bacterial endotoxin.

The Effect of Acclimatization on Tryptophan Pyrrolase Activity

As the data of Table III show, acclimatization for one week to either 5° C or 37° C has little influence on the activity of tryptophan pyrrolase and its inducibility. The data presented in this Table agree very closely with those shown in Table I for acutely exposed mice.

## IV

## DISCUSSION

The primary effect clearly established by these results is the interference by endotoxin with the thermoregulatory mechanism of the mouse. This effect is surprisingly rapid, since profound changes in body temperature of mice exposed to the various environmental temperatures become evident within a period of 30 minutes after the injection of endotoxin. At the extremes of environmental temperature employed in these experiments, i.e. at 5° C, 15° C, and 37° C, the body temperature of poisoned mice becomes severely altered and deaths ensue within a period of four hours. Deaths at the more usual environmental temperatures, 25° C - 30° C, rarely occur before 18 hours after an $LD_{50}$ of endotoxin. There is the suggestion, therefore, that endotoxin sensitizes mice to cold and to heat rather than cold and heat sensitizing mice to endotoxin. Not only can one support this idea by the difference in survival time after the $LD_{50}$ and the marked changes in body temperature,

TABLE III

Cortisone Induction of Liver Tryptophan Pyrrolase Activity Four Hours after Acute Exposure to 5° C or 37° C in Mice Previously Acclimatized for a Period of One Week at the Respective Temperature. Each assay was carried out with and without the addition of the cofactor, heme. Each value is the mean ± the standard error of the mean for the number of separate determinations shown in parentheses.

| Environmental Temperature | Tryptophan Pyrrolase Activity (μM Kynurenine/gm dry wt liver/hr) in | | | |
|---|---|---|---|---|
| | Control Mice Assayed | | Cortisone Injected Mice Assayed | |
| | Without heme | With heme | Without heme | With heme |
| 5°C | 14.8 ± 1.4 (5) | 22.9 ± 3.9 (5) | 16.5 ± 2.6 (5) | 23.4 ± 5.8 (5) |
| 37°C | 11.3 ± 1.9 (5) | 13.5 ± 2.1 (5) | 18.2 ± 2.1 (5) | 23.1 ± 3.4 (5) |

but also by the type of death that occurs. Mice at 5° C or at 37° C do not show the diarrhea, the ruffled fur, the characteristic posture and other symptoms one comes to associate with endotoxin poisoning in mice housed at 25° C.

The marked differences in $LD_{50}$ of endotoxin in mice at different environmental temperatures raises the problem of distinguishing between its primary toxicity and its secondary effect. The data presented above strongly imply that only at 30° C can one expect to detect primary responses in mice. For this reason, the apparent lack of correlation between tryptophan pyrrolase activity and its inducibility with cortisone in mice at the high and low environmental temperatures employed in these studies need not necessarily justify the conclusion that this and other enzymes play unimportant roles in endotoxin poisoning. When survival is short, the cause of death may be quite different, i.e. fatal hypothermia or hyperthermia, rather than a sequence of metabolic deficiencies that characterize primary (or subsequent) events.

One of the interesting questions that remains to be resolved and for which there is no information at present is the way in which endotoxin exerts effect

on body temperature. All attempts to indicate a penetration of the blood-brain barrier by isotopically labeled endotoxin have failed (10, 11). If endotoxin reaches the hypothalamus, it does so in such small amounts that it cannot be detected. The alternative possibility is the suggestion that endotoxin per se is not the primary mediator of the effect but rather releases a substance from the cells and tissues within the animal that in turn upsets the thermoregulatory center. The nature of this substance might be presumed to be an endogenous pyrogen (12) and yet endogenous pyrogen has properties distinct from those of endotoxin, and one would be at a loss to equate the two (13). The nature of this thermoregulatory disturbance would be most difficult to resolve in the mouse, yet the abundant evidence implicating disturbances in the peripheral vascular system (14, 15) would suggest that it is in this way, at least in part, that control of body temperature is upset. There are profound changes in energy metabolism, as suggested by the ability of certain coenzymes (6), of ATP (16), and, indeed, of aspirin (17), to protect against endotoxin. It would be of some interest to see if aspirin is equally effective in protecting against endotoxin at the different environmental temperatures. An argument in favor of the fact that endotoxin penetrates the blood-brain barrier, is the report by Kass and associates (4) that in rabbits administration of endotoxin via the carotid artery is more lethal and results in a more severe hyperthermia than endotoxin injected into the peripheral circulation. Either the endotoxin penetrates to the thermoregulatory center or else mediates this effect via a chemical release in cells situated in the central nervous system.

The series of investigations carried out under this Air Force Contract have revealed a number of interesting effects of environmental stress. Some of these are of potential practical application to man, and merit follow-up. Others are of considerable scientific interest and will be included in the symposium on environmental stress to be held in September 1965 in Kyoto, Japan, in association with the International Physiological Congress. The difficult studies that remain will require new techniques and new insights into the basic controls of body temperature. There is inadequate background of knowledge in this area and until more is learned of the fundamental nature of the process, the final solution will remain an extremely difficult one.

## REFERENCES


1. Previte, J. J. and L. J. Berry. "The effect of environmental temperature on the host-parasite relationship in mice." J. Infect. Dis. 110:201-209, 1962.

2. Previte, J. J. and L. J. Berry. "Studies on the potentiation of endotoxin in mice by exposure to cold." J. Infect. Dis. 113:43-51, 1963.

3. Berry, L. J. "Endotoxin lethality and tryptophan pyrrolase induction in cold-exposed mice." Am. J. Physiol. 207:1058-1062, 1964.

4. Kass, E. H., R. P. Atwood, and P. J. Porter. "Observations on the locus of lethal action of bacterial endotoxin." In: Bacterial endotoxins. M. Landy and W. Braun, eds. New Brunswick, N. J., Rutgers University, 1964, pp. 596-601.

5. Knox, W. E. and V. H. Auerbach. "The hormonal control of tryptophan peroxidase in the rat." J. Biol. Chem. 214:307-313, 1955.

6. Berry, L. J. and D. S. Smythe. "Effects of bacterial endotoxin on metabolism. VI. The role of tryptophan pyrrolase in response of mice to endotoxin." J. Exp. Med. 118:587-603, 1963.

7. Reed, L. J. and H. Muench. "A simple method of estimating fifty percent endpoints." Amer. J. Hyg. 27:493-497, 1938.

8. Halberg, F. "Temporal coordination of physiologic function." Cold Spring Harbor Symposia on Quant. Biol. XXV:289-310, 1960.

9. Feigelson, P. and O. Greengard. "A microsomal iron-porphyrin activator of rat liver tryptophan pyrrolase." J. Biol. Chem. 236:153-157, 1961.

10. Braude, A. I., F. J. Carey, and M. Zalesky. "Studies with radioactive endotoxin. II. Correlation of physiologic effects with distribution of radioactivity in rabbits injected with lethal doses of E. coli endotoxin labelled with radioactive sodium chromate." J. Clin. Invest. 34:858-866, 1955.

11. Herring, W., J. Herion, R. Walker and J. Palmer. "Distribution and clearance of circulating endotoxin." J. Clin. Invest. 42:79-87, 1963.

12. Atkins, E. "Pathogenesis of fever." Physiol. Rev. 40:580-646, 1960.

13. Rafter, G. W., R. D. Collins and W. B. Wood, Jr. "The chemistry of leukocytic pyrogen." Trans. Assoc. Amer. Physicians 72:323-330, 1959.

14. Zweifach, B. W. "Vascular effects of bacterial endotoxin." In: Bacterial endotoxins. M. Landy and W. Braun, Eds. New Brunswick, N. J., Rutgers University, 1964, pp. 110-117.

15. Berry, L. J. and D. S. Smythe. "Effects of bacterial endotoxins on metabolism. IV. Renal function and adrenocortical activity as factors in the nitrogen excretion assay for endotoxin." J. Exp. Med. 114:761-778, 1961.

16. Takeda, Y., Y. Miura, H. Suzuki and N. Kasai. "The elimination of metabolic disturbances produced by the injection of S. typhi, S. paratyphi B, S. flexneri and prevention of animals from death through the application of adenosinetriphosphate." Jap. J. Exp. Med. 25:133-141, 1955.

17. Smith, W. and J. H. Humphrey. "The effect of sodium salicylate upon hypersensitivity reactions." Brit. J. Exp. Path. 30:560-571, 1949.

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| | Arctic Aeromedical Laboratory<br>Fort Wainwright, Alaska |

13. ABSTRACT Acute exposure of mice to an environmental temperature of either 5° C or 37° C reduced the $LD_{50}$ of a crude Serratia marcescens endotoxin from a high of 2300 µg in mice housed at 30° C to an amount less than 40 µg. At 15° C or 32° C, the $LD_{50}$ was, respectively, 880 µg and 550 µg, while at 25° C it was 1200 µg. Control animals placed at each of these temperatures were able to maintain normothermia except for those at the high and low extremes where they became slightly hyperthermic and hypothermic. Following an injection of either twice the $LD_{50}$ or a dose of 1000 µg, the thermoregulatory ability was upset at all temperatures except 30° C. Mice at temperatures below 30° C became progressively more hypothermic as the environment was increasingly cold and vice versa at higher temperatures. It is believed that endotoxin sensitizes mice to heat and cold rather than these temperatures sensitizing to endotoxin. After one week of acclimatization at 5° C or 37° C, the $LD_{50}$ of endotoxin increased, respectively, to 790 µg and 260 µg. Inducibility of the liver enzyme tryptophan pyrrolase, believed to play a role in an animal's response to endotoxin, was evaluated at each environmental temperature. Only at the extremes was it suppressed.

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| 14. KEY WORDS | LINK A | | LINK B | | LINK C | |
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| potentiation of endotoxin lethality<br>extremes in environmental temperatures<br>thermoregulatory disturbance<br>endotoxine poisoning<br>cortisone<br>tryptophan pyrrolase activity | | | | | | |

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